**CLAIMS:**

**1.** (original) An auto-focusing method for determining an in-focus position of a sample supported on one surface of a substrate plate made of a material transparent with respect to incident electromagnetic radiation, the method utilizing an optical system capable of directing incident electromagnetic radiation towards the sample and collecting reflections of the incident electromagnetic radiation which are to be detected, and comprising:

(i) locating a focal plane of an objective lens arrangement at a predetermined distance from a surface of the substrate, which is opposite to said surface supporting the sample;

(ii) providing continuous displacement of the focal plane relative to the substrate along the optical axis of the objective lens arrangement, while concurrently directing the incident radiation towards the sample through the objective lens arrangement to thereby focus the incident radiation to a location at the focal plane of the objective lens arrangement; and

(iii) continuously detecting reflected components of the electromagnetic radiation collected through said objective lens arrangement, said detected reflected components being characterized by a first intensity peak corresponding to an in-focus position of said opposite surface of the substrate, and a second intensity peak spaced in time from the first intensity peak and corresponding to an in-focus position of said sample-supporting surface of the substrate, thereby enabling imaging of the sample when in the in-focus position of the sample-supporting surface of the substrate.

**2.** (original) The method according to Claim 1, wherein said incident radiation is in a spectral range incapable of causing luminescent response of the sample having luminescent labels therein.

**3.** (currently amended) The method according to Claim 1 ~~or 2~~, wherein said displacement is started from a certain distance between the focal plane and said opposite surface of the substrate.

**4.** (currently amended) The method according to ~~any one of preceding Claims,~~ Claim 1, wherein the displacement of the focal plane is slowed upon detecting said first intensity peak.

**5.** (currently amended) The method according to ~~any one of the preceding Claims, and also~~ Claim 1, comprising providing a relative displacement between the optical axis of the objective lens arrangement and the substrate in a plane perpendicular to said optical axis, to thereby provide intersection of the optical axis with successive locations of the substrate in said plane, and repeating steps (ii) and (iii) with respect to the successive locations of the substrate.

**6.** (currently amended) The method according to ~~any one of preceding Claims,~~ Claim 1, wherein a numerical aperture of the incident beam propagation towards the objective lens arrangement is such as to fill a back aperture defined by the objective lens arrangement.

**7.** (currently amended) The method according to ~~any one of preceding Claims,~~ Claim 1, comprising spatially separating the incident and reflected radiation components, to allow detection of the reflected radiation.

**8.** (currently amended) The method according to ~~any one of preceding Claims,~~ Claim 1, comprising passing the collected reflected radiation, propagating towards a detector, through a pinhole, thereby filtering out radiation components reflected from out of focus locations and sharpening the detected intensity peak corresponding to the light components reflected from the in-focus locations.

**9.** (currently amended) The method according to ~~any one of the preceding Claims,~~ Claim 1, comprising imaging the sample when in the in-focus position of the sample-supporting surface of the substrate.

**10.** (currently amended) The method according to ~~Claims 2 and 9,~~ Claim 2, wherein said imaging comprises irradiating the sample having luminescent labels by incident electromagnetic radiation of a wavelength range capable of exciting a luminescent response of the sample, detecting said luminescent response by an imaging detector, and generating data indicative thereof.

**11.** (original) The method according to Claim 10, wherein said incident exciting radiation is directed to the sample and the luminescent response is directed to the imaging detector, through said objective lens arrangement.

**12.** (original) The method according to Claim 11, further comprising spatially separating the luminescent response and the reflected components to allow detection of the luminescent response of the sample by said imaging detector and prevent the reflected components from reaching the imaging detector.

**13.** (currently amended) The method according to ~~any one of preceding Claims~~ Claim 1, further comprising:

- performing a sweeping-focus acquisition mode continuously acquiring images of successive planes of the three-dimensional sample along the optical axis of the objective lens arrangement, by continuously displacing the focal plane of the objective lens arrangement through the sample, starting at the sample-supporting surface of the substrate, thereby obtaining data representation of the two-dimensional projection of the three-dimensional sample onto a two-dimensional pixel array of a detector;
- processing said data representation by carrying out a two-dimensional deconvolution thereof with a predetermined Point Spread Function of a

sweeping-focusing acquisition mode, thereby obtaining a deblurred image of the two-dimensional projection of the three-dimensional sample.

**14.** (currently amended) The method according to ~~Claims 2 and 13,~~ Claim 2, wherein the sweeping focus acquisition mode comprises continuously irradiating the sample having luminescent labels therein with incident exciting radiation causing luminescent response of the sample, and continuously detecting the luminescent responses coming from the successive planes of the sample.

**15.** (original) An auto-focusing device for determining an in-focus position of a sample supported on the surface of a substrate plate made of a material transparent with respect to incident electromagnetic radiation, the device comprising:

- a light source generating a beam of the incident radiation of a predetermined wavelength range;
- a focusing optics including an objective lens arrangement;
- a light directing assembly, which is operable for directing the incident beam towards the sample through the objective lens arrangement with a predetermined numerical aperture of beam propagation to irradiate a location on the focal plane of the objective lens arrangement, and for directing reflections of said incident radiation collected by said objective lens arrangement to a detector unit, which is operable to detect said reflections and generate data indicative of their intensities;
- a drive assembly operable to provide continuous relative displacement between the focal plane of said objective lens arrangement and the substrate along the optical axis of the objective lens arrangement; and
- a control unit for operating said drive assembly to provide said continuous relative displacement and instantaneous stop of the displacement, for operating said light source and said detector to allow continuous detection of said reflections during the displacement of the focal plane, said control

unit comprising a processing device operable to be responsive to said data generated by the detector unit to identify a first intensity peak corresponding to the in-focus position of the surface of said substrate opposite to the sample-supporting surface of the substrate, and identify a second intensity peak spaced in time from the first intensity peak and corresponding to the in-focus position of the sample-supporting surface of the substrate, and to generate an auto-focusing signal upon detecting said second intensity peak.

**16.** (original) The device according to Claim 15, wherein said predetermined wavelength range of the incident radiation is incapable of causing luminescent response of the sample having luminescent labels therein.

**17.** (currently amended) The device according to Claim 15 ~~or 16~~, wherein said light directing assembly comprises a beam splitter for spatially separating between the incident and reflected radiation components, and allowing propagation of the reflected radiation components towards the detection unit.

**18.** (currently amended) The device according to ~~any one of Claims 15 to 17,~~ <u>Claim 15,</u> wherein the light directing assembly comprises a beam expander for providing said predetermined numerical apertures of the incident beam propagation to fill a back aperture defined by the objective lens arrangement.

**19.** (currently amended) The device according to ~~any one of Claims 15 to 18,~~ <u>Claim 15,</u> wherein said detection unit comprises a pinhole accommodated in the path of the collected reflected radiation propagating to a detector.

**20.** (currently amended) The device according to ~~any one of Claims 15 to 19,~~ <u>Claim 15,</u> wherein said control unit is operable to slower the displacement of the focal plane, upon detecting said first intensity peak.

**21.** (currently amended) The device according to ~~any one of Claims 15 to 20,~~ <u>Claim 15,</u> wherein said control unit is operable to actuate an image acquisition mode

to acquire images of the sample when in the in-focus position of the sample-supporting surface of the substrate.

**22.** (original) The device according to Claim 21, wherein the control unit is connectable to an imaging device operable to carry out said image acquisition mode.

**23.** (currently amended) The device according to ~~any one of Claims 15 to 20,~~ Claim 15, wherein said control unit is operable to actuate the drive assembly to provide a continuous displacement of the focal plane relative to the substrate through successive planes of the substrate along the optical axis of the objective lens arrangement starting from the sample-carrying surface, and to actuate an image acquisition mode to continuously acquire images of the successive planes of the sample.

**24.** (original) The device according to Claim 23, comprising a processing device for processing data indicative of said images to obtain a two-dimensional projection of the three-dimensional sample.

**25.** (original) An imaging system comprising the auto-focusing device of Claim 15, and an imaging device utilizing said objective lens arrangement and defining an imaging channel for electromagnetic radiation propagation towards and away from the objective lens arrangement separated from the auto-focusing channel defined by the auto-focusing device, said imaging device comprising a light source generating incident radiation and an imaging detection unit.

**26.** (original) The system according to Claim 25, wherein said predetermined wavelength range of the incident radiation used in the auto-focusing device is incapable of causing luminescent response of the sample having luminescent labels therein, said imaging device comprising a light source generating incident exciting radiation to excite a luminescent response of the sample, said response being collected by the objective lens arrangement and directed to the imaging detection unit.

**27.** (original) The system according to Claim 26, comprising a wavelength selective beam splitter accommodated in the paths of the collected reflected radiation and the collected luminescent response to provide said spatial separation between the radiation components propagating through the imaging and auto-focusing channels.

**28.** (original) A method for obtaining an image of a three-dimensional sample in the form of a two-dimensional projection of the sample, which is supported on a substrate plate made of a material transparent for incident radiation, the method comprising the steps of:

(a) providing data representative of a two-dimensional projection of images of successive planes of the sample along the optical axis of an objective lens arrangement, which are continuously acquired by a two-dimensional pixel array of a detector during a relative displacement between the focal plane of the objective lens arrangement and the sample along the optical axis of the objective lens arrangement through the sample;

(b) processing said data by carrying out a two-dimensional deconvolution thereof with the predetermined Point Spread Function of an imaging system, thereby obtaining said image in the form of the two-dimensional projection of the three-dimensional sample.